

Agronomic biofortification with magnesium nanofertilizer and its effect on the nutritional quality of beans

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Abstract

Crop quality has been compromised due to nutrient deficiencies. The macronutrient magnesium (Mg) is essential; however, it has not been considered in agronomic fertilization programs, affecting human health. The objective of the study was to increase the Mg content in the fruits of green beans cv. 'Strike' by applying Mg-nanofertilizer, as well as evaluating its effect on growth, performance and nutritional quality, versus magnesium sulfate (MgSO₄). The experiment was carried out under shade mesh conditions in Delicias, Chihuahua, Mexico during the period August-October 2022. A completely randomized experimental design was used, with two Mg sources: Mg nanofertilizer (NanoMg) and MgSO₄ at doses of 50, 100 and 200 ppm and a control without application, forming seven treatments with six repetitions each. The results indicate that the maximum yield was provided by NanoMg and MgSO₄ at 200 ppm, with values greater than 300%. The Mg distribution pattern for the NanoMg treatments presented the following concentration order: root>leaf>stem>fruit; while, for the MgSO₄ treatments it was: leaf>root>stem>fruit. The most efficient treatment in increasing the Mg content in the fruit was NanoMg at 200 ppm, which achieved a biofortification of more than 120% with respect to the control. Therefore, when consuming 100 g of green beans cv. 'Strike' biofortified by NanoMg, the recommended daily needs of the human being could be satisfied. Finally, it is concluded that nanofertilizers are the best option for a biofortification program since they offer a sustainable alternative by increasing productivity and quality in green bean fruits.

Keywords: biofortification; magnesium; nanofertilizer; nanotechnology; *Phaseolus vulgaris* L.

Introduction

Magnesium (Mg) deficiency is a widespread problem in humans, and in turn a little-recognized problem in the world (DiNicolantonio *et al.*, 2018). The lack of Mg has adverse effects on health, increasing the risk of cardiovascular and pathological diseases, predisposition to diabetes and cancer (Barbagallo *et al.*, 2021). This problem is the result of an inadequate average dietary intake, derived from the significant decrease in macro

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and micronutrients in food, as a consequence of their high deficiency in agricultural soils (DiNicolantonio *et al.*, 2018; Shinde *et al.*, 2018). Currently, Mg is called “the forgotten element”, since it is not determined or monitored in patients (Fiorentini *et al.*, 2021). In addition, it is not considered in agronomic fertilization programs, despite various studies show that Mg improves crop productivity and quality (Yan and Hou, 2018; Lu *et al.*, 2020; Wang *et al.*, 2020).

A novel strategy to combat nutrient deficiency is crop biofortification. This consists of increasing the concentrations of critical elements in the edible part of the plants, thus managing to produce crops of high nutritional value (Buturi *et al.*, 2021). However, conventional fertilizers that are used for biofortification purposes have sizes of more than 100 nanometers, so they are easily lost by sublimation and leaching, in addition to bringing a high environmental impact (Elemike *et al.*, 2019). To solve this problem, nanotechnology is the most feasible and efficient way, by providing solutions to the drawbacks of agronomic biofortification. This science offers nanofertilizers, which are products composed of nanoparticles that improve nutritional efficiency in plants, being effective in low proportions (due to their ability to penetrate biological barriers), favoring sustainable development, with a comparatively simpler method than others and potentially adequate to obtain immediate results (Echeverría-Machado, 2019; Majumdar and Keller, 2021).

A novel strategy to combat nutrient deficiency is crop biofortification. This consists of increasing the concentrations of critical elements in the edible part of the plants, thus managing to produce crops of high nutritional value (Buturi *et al.*, 2021). However, conventional fertilizers that are used for biofortification purposes have sizes of more than 100 nanometers, so they are easily lost by sublimation and leaching, in addition to bringing a high environmental impact (Elemike *et al.*, 2019). In recent years, several studies have been reported where nanofertilizers have provided positive effects on various crops. Some of them indicate that the application of Mg nanofertilizers allow greater mobility of nutrients and absorption capacity (Delfani *et al.*, 2014). In addition, they generate a greater amount of biomass and yield in bean plants. For their part, Ciscomani-Larios *et al.* (2021) report an increase in bioactive compounds and antioxidant capacity in green beans.

The common bean (*Phaseolus vulgaris* L.) is a globally important staple food due to its wide distribution and high nutritional value. This legume represents 36% of the daily protein intake of the Mexican population (Anaya *et al.*, 2021), in addition to being low in fat, rich in fiber, vitamins, bioactive compounds, and minerals (Teixeira-Guedes *et al.*, 2019). This crop with the application of nanofertilizers can be used as a vehicle to reduce Mg deficiency in people. This element is the fourth most common mineral in the human body; it is essential for health, and necessary for the functioning of more than 300 enzymes (DiNicolantonio *et al.*, 2018); plays an important part in many processes carried out by the body in the physiological function of the brain, heart and skeletal muscles, nervous system, blood sugar levels and blood pressure; it also has anti-inflammatory properties, helps form protein, bone mass and DNA, and acts as a Ca^{2+} ion antagonist (de Baaij *et al.*, 2015). On the other hand, the adequate absorption of Mg by plants is important, since it is key in their growth and development, as it plays a crucial role in the manipulation of compounds such as ATP, RNA and DNA; modulate ionic currents; be the central atom of the chlorophyll molecule; serve as a cofactor for many enzymes; among other.

Despite the reported benefits, the number of studies on the influence of biofortification with Mg nanofertilizers on growth, production, and nutritional quality in beans are limited. Therefore, the objective of the present investigation was to increase the Mg content in the fruits of green beans cv. ‘Strike’ by applying Mg nanofertilizers, as well as evaluating its effect on growth, performance and nutritional quality in contrast to the application of magnesium sulfate (MgSO_4).

Materials and Methods

Crop management and experimental design

The experiment was carried out under shade mesh conditions in the municipality of Delicias, Chihuahua, Mexico during the months of August-October 2022. Four seeds of green beans (*Phaseolus vulgaris* L.) cv. 'Strike' were sown in 13.4 L capacity plastic pots, filled with vermiculite and perlite substrate in a 2:1 ratio. After germination, only two plants per pot were left. The plants were irrigated every third day with 500 mL per pot of complete nutrient solution composed of macro and micronutrients with the following formulation: 6 mM NH_4NO_3 , 1.6 mM K_2HPO_4 , 0.3 mM K_2SO_4 , 4 mM CaCl_2 , 1 μM ZnSO_4 , 5 μM Fe-EDDHA, 2 μM MnSO_4 , 0.25 μM CuSO_4 , 0.3 μM Na_2MoO_4 , 0.5 μM H_3BO (Sánchez *et al.*, 2006); maintaining a pH of 6.0-6.1 and an electrical conductivity of 1.938 dS m^{-1} . Once the flowering stage arrived, approximately 30 days after sowing, irrigation was increased to 1000 mL per pot, due to the greater demand of the growing plant.

A completely randomized experimental design was used, with two sources of Mg: Mg nanofertilizer (NanoMg) and magnesium sulfate (MgSO_4) at doses of 50, 100 and 200 ppm (Ciscomani-Larios *et al.*, 2021), as well as a control without application, generating a total of seven treatments with six repetitions each. Finally, there were 42 experimental units with two plants each (Figure 1). The treatments were applied via the foliar route, before sunrise (6 a.m.) and at the beginning of each phenological stage of the crop (first pair of true leaves, pre-flowering, flowering, pod formation) (Table 1).

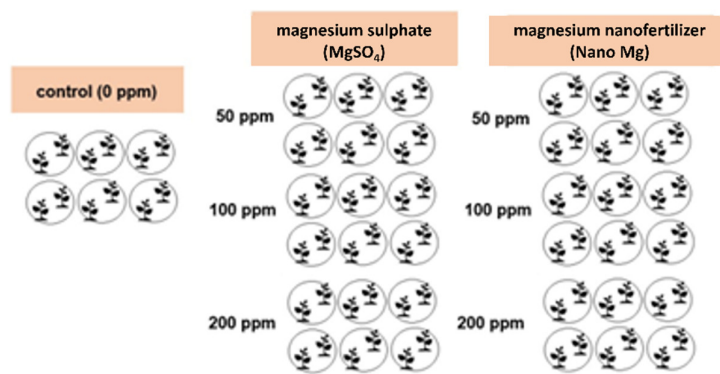


Figure 1. Experimental design and treatments

Table 1. Description of the treatments: source and dose

Mg Source	Doses (ppm)	Repetitions	Clue
Control	0	6	0
Magnesium Sulphate	50	6	50 MgSO_4
Magnesium Sulphate	100	6	100 MgSO_4
Magnesium Sulphate	200	6	200 MgSO_4
Magnesium Nanofertilizer	50	6	50 NanoMg
Magnesium Nanofertilizer	100	6	100 NanoMg
Magnesium Nanofertilizer	200	6	200 NanoMg

Plant sampling

Sixty days after the germination of the green bean seeds cv. 'Strike' and upon arrival of physiological maturity, samples were taken. The plants were sectioned in root, stem, leaf and fruit. The material was subjected

to a triple washing, first with running water to eliminate environmental residues, then two more rinses with distilled and tri-distilled water.

Plant analysis

Total biomass and yield

The plant material was introduced into a drying oven (Shell) at a temperature of 70 °C and until completely dry (24 h). Subsequently, the biomass production in dry weight was determined separately for each plant organ (leaf, stem, fruit and root), using an analytical balance (AND HR-120, San José, California, USA). The total biomass was obtained with the sum of the dry weights of each organ analyzed, reporting as biomass in plant grams of dry weight (g plant^{-1} d.w.).

The yield was obtained with the average weight of fresh pods per plant. The total production was expressed as grams per plant of fresh weight (g plant^{-1} f.w.).

Determination of nutritional quality (macro and micronutrients)

The P, K, Ca, Mg, Fe, Mn, Zn and Cu, were determined according to Wolf's (1982) methodology. For this, one gram of dry sample of nut fruit was weighed and 25 mL of triacid mixture of H_2SO_4 , HCl and HNO_3 (2.2%, 8.9% and 88.9% respectively) were added. After that, they were placed in a digester oven at 300 °C. The resulting sample was adjusted to 50 mL with deionized water (main sample). The mineral reading was performed using atomic absorption spectrophotometry (Aas, iCE 3000 Series, Thermo Scientific, Waltham, MA, USA.). In the case of the macronutrients K, Ca and Mg, a 1:100 dilution was made for reading.

The total P concentration was determined following the ammonium metavanadate (NH_4VO_3) method. 0.5 mL of the main sample was taken to which one mL of phosphorus reagent plus 3.5 mL of distilled water was added, shaken, and left to stand for one hour for later reading. The reading was performed in a spectrophotometer (Genesis 10s UV/Vis, Thermo Scientific, Waltham, MA, USA.), at 430 nm against a K_2HPO_4 standard curve. The P concentration was expressed as a percentage.

N and % protein was determined using the methodology proposed by Calvo *et al.* (2008), with the Flash 2000 team (Thermo Scientific, Waltham, MA, USA).

Magnesium content and distribution pattern

The Mg concentration was determined by atomic absorption using the iCE 3000 SERIES spectrophotometer (Thermo SCIENTIFIC). For this, 1 g of a sample of dry material was subjected to a mineralization process with nitric acid and hydrogen peroxide (Wolf, 1982), then it was measured in the Atomic Absorption Spectrophotometer at a wavelength of 285.2 nm. The concentration of Mg was expressed as mg/100 g of dry weight.

The distribution pattern is an indicator used to determine the distribution of nutrients in the different organs of the plant. For this, the Mg concentration in each organ of the green bean plants was analyzed, these concentrations were added by treatments. Subsequently, a total was determined, which represented one hundred percent and, finally, based on the Mg concentration in each of the organs, the percentages were obtained to average them according to the sources of application.

Biofortification degree of Mg in bean pods

The degree of biofortification was expressed as a percentage and was determined according to the following formula:

$$BD = \left(\frac{MgF * 100}{MgC} \right) - 100$$

Where:

BD: Biofortification degree

MgF: Mg content in the fruit of plants with foliar application of Mg

MgC: Mg content in the fruit of the control treatment

Statistical analysis

The data obtained were subjected to an analysis to evaluate the normality and homogeneity of variance and later an analysis of variance ($\alpha= 0.05$) was performed. When there were significant differences, an LSD mean separation test was performed with a significance of 95%. In addition, a Pearson correlation analysis was performed using the statistical package SAS version 8 software (SAS, 2004).

Results and Discussion

Total biomass and yield

Mg has an important impact on crop development and productivity (Wang *et al.*, 2020). In the present study, the highest accumulation of total biomass was observed in the MgSO₄ treatment at 200 ppm, with an increase of 100% with respect to the control without application (Figure 2). Significant increases can be seen from doses of 50 ppm for MgSO₄ and for NanoMg, only at high doses (Figure 2). These results agree with those reported by Neuhaus *et al.* (2014), where the foliar application of MgSO₄ at 200 mM in wheat plants significantly increased yield per pot. For NanoMg treatments, an increasing trend was observed as the dose increased.

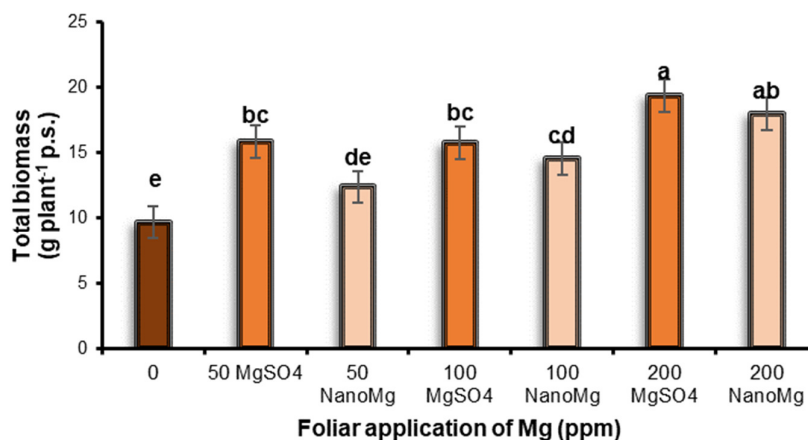


Figure 2. Effect of foliar application of MgSO₄ and NanoMg on green bean plants cv. 'Strike' on total biomass in dry weight. Different letters show statistically significant differences according to LSD test ($P < 0.05$)

Mg fertilization improves crop yield, so its application is an important measure to boost crop production (Wang *et al.*, 2020). In the present experiment, the 200-ppm dose of NanoMg stood out (Figure 3), surpassing the control in 392% in yield, without being statistically different from the 200-ppm dose of MgSO₄. These results agree with the trends for pod biomass (Figure 2), with a positive correlation ($r=0.6796$, $p<0.0001$), in addition to exceeding the national average determined for arid zones by Salinas-Ramírez *et al.* (2012), by 54%. Significant increases can be seen with respect to the control from low doses (50 ppm), with increases of more than 100%.

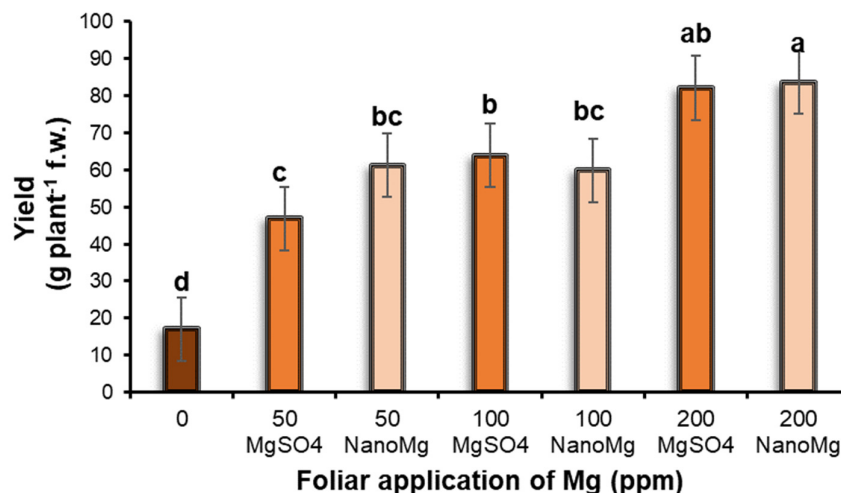


Figure 3. Effect of foliar application of MgSO₄ and NanoMg on green bean plants cv. 'Strike' on fresh weight performance. Different letters show statistically significant differences according to LSD test ($P < 0.05$).

The increase in yield due to the application of NanoMg at 200 ppm, is due to the importance of the Mg element, as a central element of chlorophyll, it is essential for the metabolism of Carbon (C) and N. Its presence promotes the synthesis of chlorophyll and increases the activity of Rubisco, the enzyme in charge of fixing CO₂, therefore, an increase in the rate of photosynthesis. On the other hand, Mg also has a direct and positive effect on the efficient use of N by regulating the absorption, assimilation and distribution of N. As a direct consequence, the rates of formation of photosynthetic products increase, which are reflected in greater biomass and yield ratios. Also, the application of Mg can improve the levels of plant growth hormones in the plant (Mahawa *et al.*, 2017; Hauer-Jákli and Tränkner, 2019; Khodadadi *et al.*, 2021). Finally, various studies suggest that the supply of Mg at the nanoscale is more easily absorbed by plants (Echeverría-Machado, 2019).

Nutritional quality

Nutritional quality is a highly important variable that must be considered, since it establishes the functionality of food in the diet (Hermosillo, 2012). The productivity of a crop is related to an appropriate supply of N. In plants, the final products of N assimilation are proteins, which are necessary for adequate growth of the human body. The results of this investigation did not present differences in the accumulation of N in the pods with respect to the control (Table 2). Despite this, in all doses and in both forms of application, a decline of up to 5% was observed in comparison with the control, as the dose increased. In the doses that found the highest yield (NanoMg and MgSO₄, 200 ppm), they were the ones with the lowest concentration of said nutrient, possibly due to the energy expenditure in the production of pods. These results are related to what was mentioned by Karooki *et al.* (2021), for the potato crop, where the applications of higher amounts of Mg led to a decrease in the N content. Also, they are like those found by Kleiber *et al.* (2012), who report that the N accumulated in onion bulbs after the application of Mg at doses of 50 g Mg m⁻², was higher than for higher doses. In the case of proteins, significant differences were found, observing a decrease in their content with respect to the control, as the Mg dose increased. This agrees with what was indicated for potato tubers, where Mg nutrition, either as a foliar or soil treatment, did not have a significant influence on the protein content Karooki *et al.* (2021). Said drop was less marked for the treatments with MgSO₄ compared to NanoMg. This may be due to the sulfate ion; sulfur supports the use of N, while stimulating yields (Zlámálová *et al.*, 2016).

For phosphorus, significant differences were found ($p \leq 0.05$), observing a decrease with respect to the control for the NanoMg treatments, although without being statistically significant. While, for $MgSO_4$, the values exceeded the control, although they did differ statistically. These data are equal to what was mentioned for the wheat crop, where the application of Mg had a positive influence on the absorption of P by the plant (Rathore and Tarafdar, 2015).

Regarding potassium (K), highly significant differences were found ($p < 0.001$), highlighting the $MgSO_4$ treatments with 12-17% compared to the control. On the other hand, for NanoMg, increases are observed only when applying high doses (200 ppm) of 10% with respect to the control (Table 2). This is related to what was mentioned by Karooki *et al.* (2021), who report a positive effect of increasing Mg nutrition on K in the potato crop.

Table 2. Concentration of macronutrients in the bean fruit cv. 'Strike' after the application of magnesium sulfate ($MgSO_4$) and magnesium nanofertilizer (NanoMg)

Doses (ppm)			Concentration (g 100 g ⁻¹ dry weight)			
NanoMg	N	P	K	Ca	Mg	Protein %
0	3.643 a	0.208 abc	3.233 c	0.433 ab	0.241 e	28.21 a
50	3.550 a	0.193 abc	3.280 c	0.497 a	0.427 cd	22.07 a
100	3.552 a	0.183 bc	3.221 c	0.481 a	0.438 bc	17.96 b
200	3.446 a	0.134 c	3.568 b	0.444 ab	0.526 a	17.63 b
$MgSO_4$	N	P	K	Ca	Mg	Protein %
0	3.643 a	0.208 abc	3.233 c	0.433 ab	0.241 e	28.21 a
50	3.555 a	0.320 a	3.705 ab	0.433 ab	0.414 d	21.92 ab
100	3.542 a	0.231 abc	3.625 ab	0.381 b	0.445 bc	21.23 b
200	3.518 a	0.292 ab	3.782 a	0.483 a	0.455 b	20.78 b
Significance	NS	*	***	*	*	*

*Different letters show statistically significant differences (LSD Test, $P \leq 0.05$).

In the case of the mineral calcium (Ca), differences were found, appreciating increases with respect to the control with both sources of Mg, although without being significant (Table 2). Although, multiple studies report the opposite, accentuating the antagonistic effect between Mg and Ca, where Ca gradually decreases as Mg increases (Kleiber *et al.*, 2012; Lu *et al.*, 2020). However, there is also research that found a positive relationship between both nutrients, such as Karooki *et al.* (2021), in potato or onion crops (Kleiber *et al.*, 2012).

On the other hand, in the micronutrient zinc (Zn), it is appreciable that there is a decrease with respect to the control of up to 14%, as the application dose increases (Table 3). This agrees with the results for the pepper crop (Lu *et al.*, 2020) and for the wheat crop (Rathore and Tarafdar, 2015).

Copper (Cu) is a redox active transition element with roles in photosynthesis, respiration, C and N metabolism, and protection against oxidative stress (Marschner, 2012). In relation to this mineral, it is appreciable that when the dose of Mg from both sources increases, its absorption is favored with respect to the control, although this is only significant after applying $MgSO_4$ at a dose of 200 ppm, differing from the control by 37% (Table 3). These data agree with what was reported by Rathore and Tarafdar (2015), for the wheat crop.

The manganese ion (Mn), significant differences were found, presenting a decrease as the Mg dose with both sources increased (Table 3). This may be due to the antagonistic effect between both ions (Marschner, 2012).

Regarding iron (Fe), significant differences were found, appreciating an increase with respect to the control when applying both sources of Mg (Table 3). These results coincide with what was found by Rathore

and Tarafdar (2015), where the application of Mg had a positive influence on the absorption of said nutrient in the wheat crop.

Some authors mention that Mg doses beyond those required for maximum yield rarely induce a greater improvement in product quality (Zlámálová *et al.*, 2016). However, Marschner (2012) indicates that, generally, high concentrations of Mg improve nutritional quality. For this work, it was observed that certain minerals were benefited (such as K or Cu), which may be due to the role of Mg in regulating ion transport pathways (Shaul, 2002).

Table 3. Concentration of micronutrients in the bean fruit cv. 'Strike' after the application of magnesium sulfate (MgSO₄) and magnesium nanofertilizer (NanoMg)

Doses		Concentration (g 100 g ⁻¹ de dry weight)		
Nano Mg	Zn	Cu	Mn	Fe
0	29.000 b	12.250 c	56.583 a	72.000 b
50	20.75 d	11.833 c	46.333 b	82.167 ab
100	22.92 cd	12.417 bc	48.167 ab	73.417 b
200	24.50 c	12.583 bc	44.250 b	90.500 a
MgSO ₄	Zn	Cu	Mn	Fe
0	29.000 b	12.250 c	56.583 a	72.000 b
50	25.417 c	14.500 b	47.167 b	92.167 a
100	33.500 a	13.833 bc	56.500 a	76.083 b
200	25.000 c	16.750 a	48.500 ab	84.833 ab
Significance	***	**	*	*

*Different letters show statistically significant differences (LSD Test, $P \leq 0.05$).

Magnesium content and distribution pattern

For plants to acquire and maintain high Mg concentrations, a highly efficient Mg transport system is necessary for its uptake, storage and translocation (Chen *et al.*, 2018). The results of this study showed significant differences, observing an increase in said element with the doses of MgSO₄ and NanoMg (Table 4). The highest concentrations in the leaf were found in the treatment with 200 ppm MgSO₄, with no statistical difference with the NanoMg treatment at 200 ppm, exceeding the control by 80 and 79%, respectively. These results agree with Nehaus *et al.* (2014), where the foliar application of 200 mM MgSO₄ on *Vicia Faba* resulted in an increase in the concentration of Mg.

Regarding the fruit, when increasing the dose in each treatment, the concentration of Mg was higher (Table 4). The highest concentration of Mg was for the NanoMg treatment at 200 ppm, surpassing the MgSO₄ treatment at 200 ppm by 29%. These data follow the trend also seen in the concentration of Mg in the leaf ($r=0.88$, $p<0.0001$), something like that reported by Wang *et al.* (2020), where a significant positive linear correlation was observed between crop yield and the concentration of Mg in the leaves of vegetables, fruits and grasses. This behavior may be because Mg has a high mobility in the phloem and the application of Mg fertilizers can efficiently increase its concentration in leaves and sinks (Buturi *et al.*, 2021).

Concerning the root, the highest concentration occurred in the treatment of NanoMg at 50 ppm, without presenting statistical differences with the dose of 100 ppm of NanoMg and MgSO₄ at 50 ppm. In both treatments, by increasing the dose, the concentration of Mg in the root decreases. This may be because Mg is delivered to different tissues of the plant with preferential distribution to developing tissues as in this case, Mg is directed towards leaves and fruits, therefore it decreases in roots (Chaudhry *et al.*, 2021). On the other hand, in the stem the highest concentration of Mg occurred in the MgSO₄ treatment, at a dose of 200 ppm.

About the total Mg concentration, the NanoMg treatment at a rate of 200 ppm stands out with a 63% increase compared to the control. These results agree with what was found by Setareh *et al.* (2021), whereby increasing the supply of Mg in *Spinacia oleracea*, its concentration in leaves increased.

In general, the values that stood out were found in the MgSO₄ and NanoMg treatments with the highest dose (200 ppm), reflected in biomass production (Figure 2), as well as in yield (Figure 3).

Table 4. Effect of foliar application of MgSO₄ and NanoMg on the Mg content in green beans cv. 'Strike'

Foliar application of Mg	Mg content (mg/100 g)									
	Leaf		Root		Fruit		Stem		Total	
Control - 0 ppm	738	d	1.130	d	241	e	398	d	2.506	d
MgSO ₄ - 50 ppm	1.130	c	1.732	b	414	d	564	b	3.840	bc
MgSO ₄ - 100 ppm	1.259	b	1.623	b	445	c	580	b	3.907	abc
MgSO ₄ - 200 ppm	1.331	a	1.282	c	455	b	643	a	3.711	c
NanoMg - 50 ppm	1.154	c	1.798	a	427	cd	599	b	3.979	ab
NanoMg - 100 ppm	1.108	c	1.708	b	438	bc	508	c	3.762	c
NanoMg - 200 ppm	1.320	ab	1.641	b	526	a	587	b	4.073	a

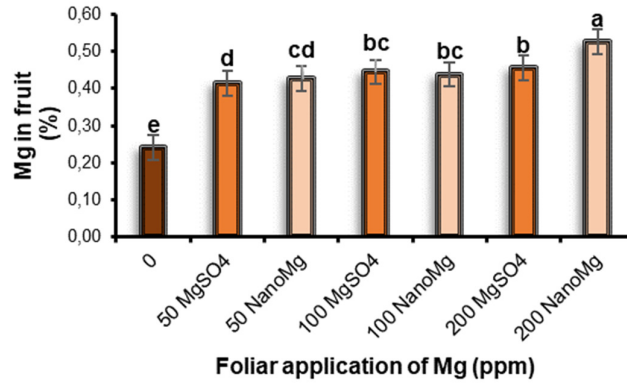
*Different letters show statistically significant differences (LSD Test, $P \leq 0.05$).

To determine the distribution of nutrients in the different organs of the plant, the distribution pattern is a good indicator (Yamaji and Ma, 2014). In the present study, the distribution pattern of Mg in bean plants presented the following distribution for NanoMg treatments: fruit (12.91 %), stem (14.40 %), root (40.98 %) and leaf (32.41 %) (Figures 4 and 5). While, for the MgSO₄ treatments, the distribution pattern was the following: fruit (12.27%), stem (17.34%), root (34.54%) and leaf (35.86%) (Figures 4 and 5).

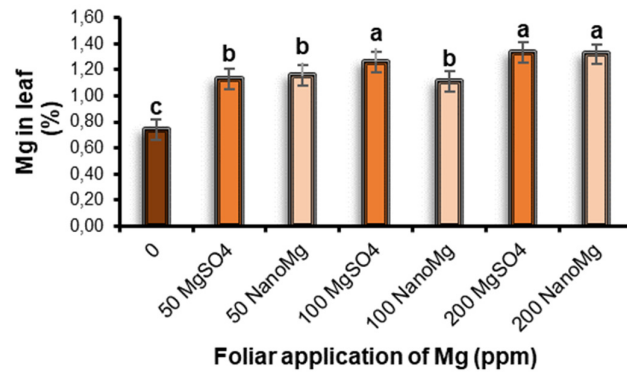
The Mg distribution pattern shows that in all the application rates studied with the NanoMg treatment, the highest concentration of magnesium occurred in the roots, followed by the leaves, then the stems and finally the fruits (R>H>T >F) (Figures 4 and 5). This is like what was reported by Hawesford *et al.* (2012), where it is mentioned that the Mg concentrations in the spruce endodermis cells were higher, followed by the mesophyll cells. Likewise, Jaghdani *et al.* (2021), report a higher concentration of Mg in the roots compared to the leaves and shoots in the spinach (*Spinacia oleracea*) crop, the same case reported for *Brassica rapa* (Blasco *et al.*, 2015).

For its part, the Mg distribution pattern at the 50 and 100 ppm application doses with the MgSO₄ treatment showed the same behavior as that seen in the NanoMg treatment (R>H>T>F). However, at a dose of 200 ppm this pattern changed, finding the highest concentration of Mg in the leaves, followed by the roots, then the stems and finally the fruits (H> R>T>F) (Figure 4 and 5). The foregoing is like what was mentioned by Mitra (2015), where it is pointed out that the total concentration of Mg in the vacuoles of the barley mesophyll is high, representing up to 7 mM. Similarly, Blasco *et al.* (2015) point out that the concentration of Mg in aerial tissues increases with high supplies of Mg in *Brassica rapa*.

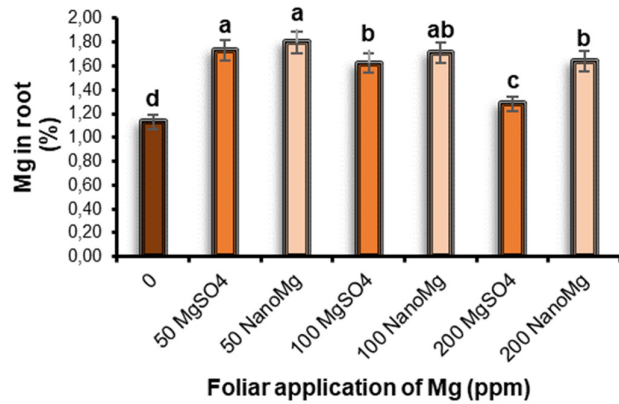
Mg concentrations were favored with both Mg sources, observing a similar distribution pattern. In addition, high dose treatments allowed a greater accumulation of biomass and yield (Figure 2 and Figure 3, respectively).



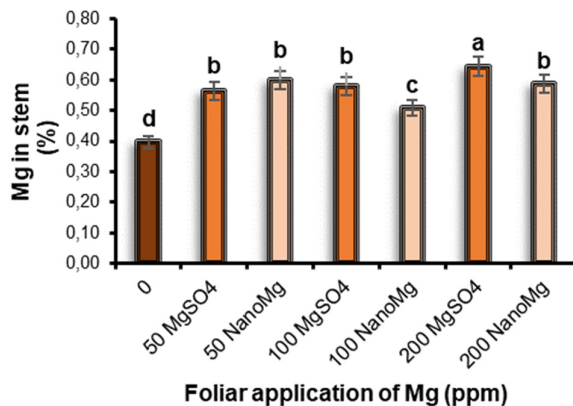
(A)



(B)



(C)



(D)

Figure 4. Effect of foliar application of the different treatments of MgSO₄ and NanoMg on the concentration of magnesium in green bean plants cv. ‘Strike’ on: A) fruit, B) leaf, C) root and D) stem. Different letters show statistically significant differences according to LSD test ($P < 0.05$)

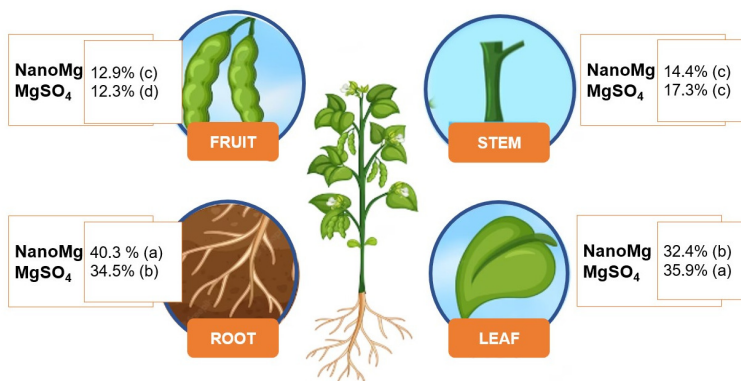


Figure 5. Mg distribution pattern (fruit, stem, root and leaf) depending on the foliar application of magnesium nanofertilizer (NanoMg) and magnesium sulfate (MgSO₄) in green beans cv. ‘Strike’. Different letters show statistically significant differences according to LSD test ($P < 0.05$)

The higher percentages of Mg accumulation in the root may be due to the fact that excess concentrations are directed to the vacuoles of the endodermal cells, in order to serve as a reserve to maintain Mg homeostasis in other cells (Hauer-Jákli and Tränkner, 2019).

Biofortification degree of Mg in bean pods

Biofortification consists of increasing the nutritional value of food crops through agronomic practices or biotechnology (Buturi *et al.*, 2021). In the present study, significant statistical differences were obtained, highlighting the NanoMg treatment at a dose of 200 ppm, increasing by 121% in relation to the control without application (Table 5). Another outstanding treatment was MgSO₄ at a dose of 200 ppm, with 92% efficiency with respect to the control (Table 5). In general, it is appreciable that, by increasing the application dose, the degree of biofortification also increases, which is appreciated with the trend observed for total biomass (Figure 2). Kumsa *et al.* (2019), indicate that the foliar application of Mg increased its content in Italian ryegrass (*Lolium multiflorum* L.), as the application rate of Mg fertilizers increased for all cultivars, with increases from 85 to 140 %, with a rate of application of 1500 μ M of MgCl₂. Tomatoes (*Lycopersicum*

esculentum L.) biofortified with Mg showed an increase in Mg content of 2.1% with respect to the control (Coelho *et al.*, 2022).

Table 5. Degree of biofortification achieved by the application of magnesium sulfate ($MgSO_4$) and magnesium nanofertilizer (NanoMg) in green beans cv. 'Strike'

Treatment	Biofortification degree (%)
Control	-
$MgSO_4$ 50	70.8 d
$MgSO_4$ 100	87.5 bc
$MgSO_4$ 200	91.7 b
NanoMg 50	79.1 cd
NanoMg 100	83.3 bc
NanoMg 200	120.8 a

*Different letters indicate significant differences (LSD Test, $P \leq 0.05$).

Based on these results, it can be deduced that the main growth indicators such as biomass and yield were favorable for the high doses of NanoMg (200 ppm). $MgSO_4$ at a dose of 200 ppm also presented favorable results, keeping up with the nanofertilizer at such a dose. However, the low doses of NanoMg and $MgSO_4$ also presented favorable results with respect to the control without application, with degrees of biofortification of more than 70%. With this it can be demonstrated that nanofertilizers can act the same and even surpass a conventional source such as $MgSO_4$. However, the response to the application of these two Mg sources may differ between crops, in addition to other abiotic factors.

The results obtained in this study exceed the concentration of Mg in seeds of native bean populations originating from four regions of Oaxaca by more than 64% (Espinoza-García *et al.*, 2016). Likewise, biofortification with Mg -even at low doses- allowed doubling the Mg concentration in green beans cv. 'Strike' in contrast to the maximum concentration reported for black beans (220 mg/100 g) and leaving 104 varieties of existing genotypes in Mexico below (Gutierrez-Ruelas *et al.*, 2018).

The RDA for Mg intake varies by life stage; however, it is within the range of 200-400 mg/day (DiNicolantonio *et al.*, 2018; Barbagallo *et al.*, 2021). Therefore, when consuming 100 g of green beans cv. 'Strike' biofortified by NanoMg and $MgSO_4$ from low doses it is possible to satisfy the daily needs of the human being. However, Armendáriz *et al.* (2016), mention that the Mg content can be reduced by up to 50 % because of cooking. Therefore, the best source to achieve biofortification with Mg is the NanoMg treatment at a dose of 200 ppm, since it is the most efficient to increase the Mg content and its presence in the fruit.

Conclusions

The most efficient doses and sources to increase the growth of green bean plants cv. 'Strike' were those of 200 ppm for NanoMg and $MgSO_4$, which favored the accumulation of total biomass and yield. Regarding the distribution pattern of Mg in bean plants, the following distribution was presented for NanoMg treatments: root>leaf>stem>fruit; while for the $MgSO_4$ treatments the distribution pattern was: leaf>root>stem>fruit. The most efficient treatment in increasing the Mg content in the fruit was NanoMg at 200 ppm, which achieved a biofortification of more than 120% with respect to the control. Therefore, when consuming 100 g of green beans cv. 'Strike' biofortified by NanoMg, the recommended daily needs of the human being could be satisfied. In conclusion, the form and dose of Mg that could improve the nutritional quality and be optimal for future biofortification programs in beans was NanoMg at 200 ppm, since it allowed a higher yield and accumulation of Mg in the fruits, in addition to being efficient for most of the determined parameters.

Authors' Contributions

E.S and A.S-M designed the study. C.A.R-E. and A.S-M. analyzed the data. E.S and A.S-M. prepared the manuscript, while S.P-A., A.S-M., and C.A.R-E conducted the experiments. A.S-M., C.A.R-E., and E.S. organized the data and performed the statistical analysis. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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